Tetrahedron Vol 44, No 20, pp 6447 to 6450, 1988
Printed in Great Britain

0040-4020/88 $3.00 + .00
Pergamon Press plc

THE REACTION OF CYSTEINE WITH 1,4-BENZOQUINONE: A REVISION.

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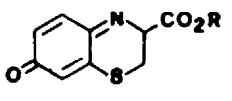
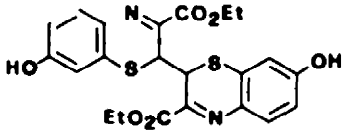
(*Received in UK 2 August* 1988)

Abstract. Under carefully controlled conditions, the reaction of L-cysteine with 1,4-benzoquinone leads to a mixture of 1,4-benzothiazine oligomers, arising by decarboxylation and subsequent polymerization of the cyclic quinonimine 1, previously reported as the reaction product. When the reaction was stopped in the early stages by addition of sodium borohydride, work up of the mixture gave, besides the reduced dimer 11, the dihydrobenzothiazine 10, consistent with the intermediacy of the 3-unsubstituted benzothiazine 5.

The reaction of cysteine with quinones plays an important role in many biological processes, which include the biosynthesis of firefly luciferin[1,2] and the formation of the sulphur-containing phaeomelanins[3] and trichochromes[4] found in red hair and feathers.
In 1944 Kuhn and Beinert[5] reported that cysteine and its ethyl ester react with 2 equivalents of 1,4-benzoquinone to give mainly the cyclic quinonimines 1 and 2, respectively. In a reexamination of these reactions[6] it was found that, in the case of cysteine ethyl ester, the reaction leads in fact to a mixture of diastereoisomers corresponding to the gross structure 3. Moreover, evidence was obtained that the reaction of 1,4-benzoquinone with cysteine proceeds differently to give a complex mixture of products, the nature of which remained elusive.



1 R = H
2 R = Et



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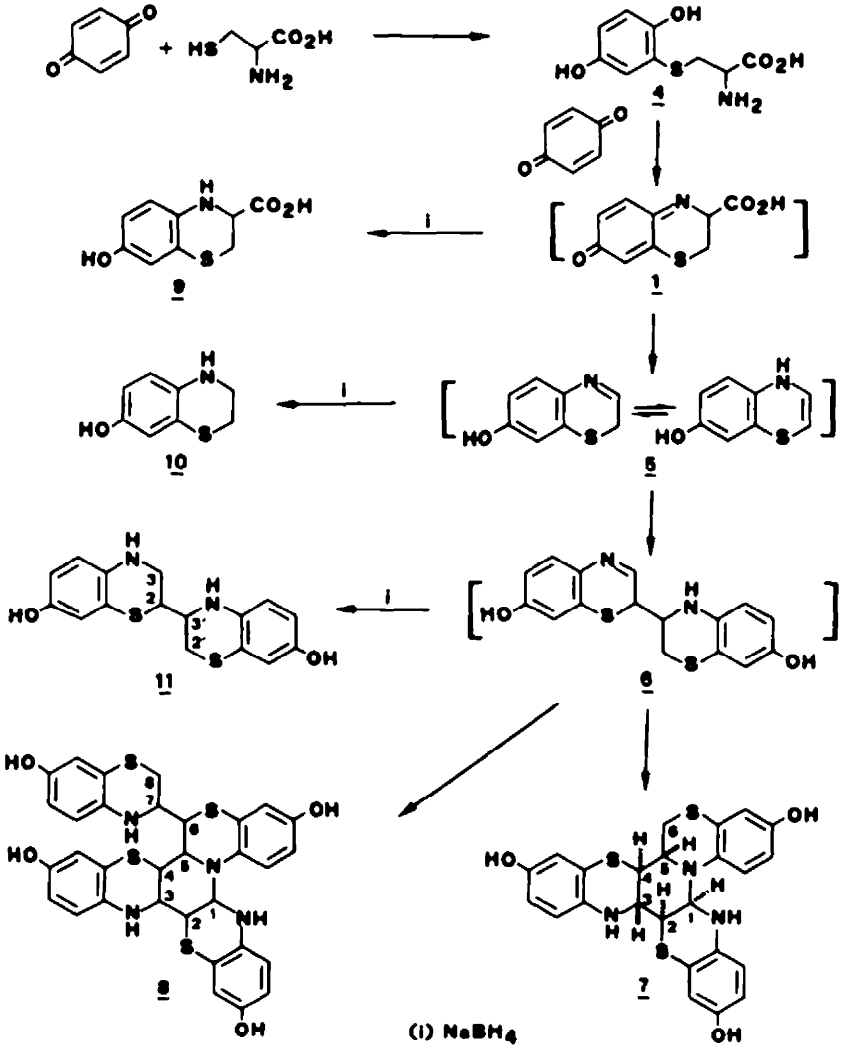
In connection with our interest in the chemistry of melanogenesis,[7] we have now reexamined the addition of 1,4-benzoquinone to L-cysteine, and have found that, when performed in the absence of air, the reaction gives, besides a small amount of the postulated intermediate adduct 4 and some polymeric material, a major colourless product, identified as the cyclic trimer 7. Fractionation of the material on silica allowed also the isolation of a smaller amount of a related tetramer which was assigned the gross structure 8.
The trimer 7 exhibits absorption maxima at 323 and 236 nm, consistent with the presence of a dihydrobenzothiazine chromophore.[8] The ^{13}C-NMR spectrum shows resonances for 18 aromatic carbons, 1 methylene and 5 methine groups, one of which is at a relatively low field (δ 68.10 ppm). The ^{1}H-NMR spectrum (acetone-d_6) shows five D_2O-exchangeable protons due to three OH and

two NH groups, a low-field doublet at δ 5.69 ppm, due to the methine linked to two nitrogen atoms, and an ABX system attributable to the cyclic CH_2-CH grouping.

The large values (9-10.5 Hz) of the vicinal coupling constants $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ are consistent with an axial-axial relationship between H-2 and H-3, H-3 and H-4, H-4 and H-5, while the value of $J_{1,2}$ (3.5 Hz) points to an equatorial position of H-1. This latter assignment was substantiated by a significant NOED effect between H-1 and the proximal aromatic proton at δ 6.99. These observations would favour the trimer with the stereochemistry depicted in 7.

As far as the structure of the tetramer 8 is concerned, it followed by straightforward analysis of the ^{1}H-NMR spectrum, differing from that of 7 mainly in the presence of additional signals attributable to a dihydro-1,4-benzothiazine unit linked at position 6 of the trimer. Attempts to define the stereochemistry of the tetramer did not give conclusive results, owing to the intrinsic complexity of the ^{1}H-NMR spectrum.

Mechanistically, the formation of 7 and 8 proceeds probably through repeated imine-enamine type condensation of the unstable 1,4-benzothiazine 5 arising by decarboxylation of the quinonimine 1.



In line with this view, when the reaction was stopped in the early stages (e.g. 1 min) by addition of sodium borohydride, work up of the mixture afforded, besides the acid 9, the dihydrobenzothiazine 10, arising evidently by reduction of 5. When sodium borohydride was added to the reaction mixture at a later time (e.g. after 15 min), the dimer 11 was also obtained, which supposedly derives by reduction of the first formed aldolization product 6.

It seems therefore that the complexity of the reaction of 1,4-benzoquinone with L-cysteine is mainly due to the tendency of the intermediate quinonimine 1 to undergo rearrangement with concomitant decarboxylation to give the 3-unsubstituted benzothiazine 5, which readily polymerizes by imine-enamine condensation.

This behaviour parallels that of the parent benzothiazine, which, as reported,[9] is a highly unstable compound, and behaves similarly to other heterocyclic enamines[10] in forming reversible aldolization products.

EXPERIMENTAL

M.ps. were determined with a Kofler hot stage apparatus and are uncorrected. UV spectra were recorded with a Perkin Elmer 550 S spectrophotometer. ^{1}H-NMR spectra (200 MHz) and ^{13}C-NMR spectra (50 MHz) were recorded on a Varian XL 200 spectrometer (δ values are referred to TMS as the internal standard). The electron impact mass spectra were determined with a Kratos MS 50 mass spectrometer. For flash chromatography, silica gel 60 Merck 9385 was used. Analytical and preparative TLC were carried out on precoated silica gel F-254 plates (0.25 and 0.50 mm layer thickness, E. Merck). Proportions for mixed solvents are by volume. The chromatograms were examined by UV irradiation at λ 366 and 254 nm, then sprayed with 2% ceric sulphate in 2N sulphuric acid. 1,4-benzoquinone and L-cysteine were purchased from Aldrich; all other chemicals were of the highest purity commercially available.

Reaction of L-cysteine with 1,4-benzoquinone.

1) Isolation of 7 and 8.

Under the reported conditions, the reaction afforded a complex mixture of chromatographically ill-defined products. After several trials, the following procedure was adopted, which gave a more definite pattern of products: to a stirred solution of L-cysteine (487 mg) in 70 ml of 0.2 M phosphate buffer, pH 6, a solution of 1,4-benzoquinone (870 mg) in 70 ml of water was added dropwise, under a stream of nitrogen, over a period of 15 min. After 30 min the reaction mixture was extracted with ethyl acetate and the combined organic layers were washed with water, dried over Na_2SO_4 and evaporated to dryness. The yellowish-brown residue (995 mg) was taken up in ether and fractionated by flash chromatography over silica gel (ether-benzene 7:3) to give two fractions (295 mg and 30 mg, respectively).

Further purification of the main fraction by PLC (ether-benzene 7:3) afforded 90 mg of 7 as a glassy, optically inactive oil, homogeneous by HPLC analysis, λ max (MeOH) 323, 236 nm; m/e 495 (M+, $C_{24}H_{21}N_3O_3S_3$: found 495.0753, requires 495.0745); ^{1}H-NMR (acetone-d_6): δ (ppm) 8.00 (1H, bs, OH), 7.75 (1H, bs, OH), 7.71 (1H, bs, OH), 6.99 (1H, d, J=8.8 Hz, aromatic proton), 6.75-6.60 (4H, m, aromatic protons), 6.55-6.40 (4H, m, aromatic protons), 5.69 (1H, d, J=3.5 Hz, H-1), 5.38 (1H, bs, NH), 4.80 (1H, bs, NH), 4.26 (1H, ddd, J=10.1, 4.8, 3.5 Hz, H-5), 3.62 (1H, dd, J=10.1, 9.0 Hz, H-4), 3.48 (1H, dd, J=10.4, 9.0 Hz, H-3), 3.20 (1H, dd, J=10.4, 3.5 Hz, H-2), 3.08 (1H, dd, J=13.8, 3.5 Hz, H-6a), 2.78 (1H, dd, J=13.8, 4.8 Hz, H-6b); ^{13}C-NMR (acetone-d_6): δ (ppm) 68.10 (d, C-1), 55.53 (d, C-5), 54.68 (d, C-3), 47.80 (d, C-4), 43.66 (d, C-2), 32.30 (t, C-6); and 18 aromatic carbons at δ 151.22 (s), 151.14 (s), 150.55 (s), 137.67 (s), 137.50 (s), 135.98 (s), 126.57 (s), 118.18 (s), 118.07 (d), 117.83 (d), 117.34 (d), 116.52 (d), 115.65 (d), 115.39 (d), 114.69 (d), 114.14 (d), 113.55 (d), 111.42 (s).

Purification of the minor fraction by PLC (ethyl acetate-benzene 7:3) afforded 7 mg of 8, λ max (MeOH) 325, 234 nm; m/e 660 (M+, $C_{32}H_{28}N_4O_4S_4$: found 660.0983, requires 660.0993); ^{1}H-NMR (acetone-d_6): δ (ppm) 8.04 (1H, bs, OH), 7.77 (1H, bs, OH), 7.70 (1H, bs, OH), 7.61 (1H, bs, OH), 7.15 (1H, d, J=9.8 Hz, aromatic proton), 6.8-6.35 (11H, m, aromatic protons), 5.93 (1H, d, J=3.5 Hz, H-1), 5.67 (1H, bs, NH), 5.40 (1H, bs, NH), 4.75 (1H, bs, NH), 4.69 (1H, dd, J=10.3, 2.4 Hz, H-5), 3.77 (1H, dd, J=10.3, 8.9 Hz, H-4), 3.7-3.5 (3H, m, H-3, H-6 and H-7), 3.46 (1H,

dd, J=12.5, 2.8 Hz, H-8a), 3.16 (1H, dd, J=10.5, 3.5 Hz, H-2), 2.94[11] (1H, dd, J=12.5, 2.6 Hz, H-8b).
TLC and HPLC analysis of the aqueous phase revealed the presence of a small amount of an o-diphenolic, α-aminoacidic compound, identified as cysteinylhydroquinone 4 by comparison with an authentic sample prepared as described by Ito.[12] In separate experiments 4 was found to be formed in almost quantitative yield after addition of 1 equivalent of 1,4-benzoquinone to L-cysteine.

2) Reduction with sodium borohydride:
a. isolation of 9 and 10.

Benzoquinone (216 mg) was allowed to react with L-cysteine (121 mg) as described above; after 1 min the mixture was reduced with excess solid sodium borohydride, acidified to pH 2 and extracted with ethyl acetate. The organic layers were washed with water, then extracted with 2% $NaHCO_3$ containing some sodium dithionite. Evaporation of the neutral fraction gave a greyish residue, which was fractionated by flash chromatography (ether) to give 39 mg of 10, prisms from ethanol, m.p. 127-128°C, λ max (MeOH) 322, 232 nm; m/e 167 (M+, C_9H_9NOS: found 167.0397, requires 167.0405); ^{1}H-NMR (CD_3OD): δ (ppm) 6.48 (1H, dd, J=8.4, 0.7 Hz, H-5), 6.44 (1H, dd, J=2.7, 0.7 Hz, H-8), 6.39 (1H, dd, J=8.4, 2.7 Hz, H-6), 3.46 (2H, AA' part of AA'BB' system, $-CH_2-N<$), 3.05 (2H, BB' part of AA'BB' system, $-CH_2-S-$); ^{13}C-NMR (acetone-d_6): δ (ppm) 150.02 (s, C-7), 136.83 (s, C-10), 117.72 (s, C-9), 117.52 (d, C-8), 114.07 (d, C-5 or C-6), 113.61 (d, C-6 or C-5), 43.00 (t, C-3), 27.34 (t, C-2).
The bicarbonate extracts were acidified to pH 2 and extracted with ether. The organic layers were washed with water, dried and evaporated to give 42 mg of 9 as an amorphous powder, λ max (MeOH) 320, 232 nm; m/e 211 (M+, $C_9H_9NO_3S$: found 211.0297, requires 211.0303); ^{1}H-NMR (acetone-d_6): δ (ppm) 6.65-6.45 (3H, m, aromatic protons), 4.36 (1H, dd, J=5.8, 3.8 Hz, H-3), 3.22 (1H, dd, J=12.0, 3.8 Hz, H-2a), 3.17 (1H, dd, J=12.0, 5.8 Hz, H-2b); ^{13}C-NMR (acetone-d_6): δ (ppm) 173.14 (s, COOH), 150.04 (s, C-7), 134.93 (s, C-10), 117.86 (d, C-8), 116.72 (s, C-9), 114.21 (d, C-5 or C-6), 114.03 (d, C-6 or C-5), 54.60 (d, C-3), 27.86 (t, C-2).

b. isolation of 11.

A solution of benzoquinone (434 mg) was added to a solution of L-cysteine (243 mg) under the usual conditions; after 15 min the mixture was reduced with excess sodium borohydride, acidified with 0.1 M HCl to pH 5.8 and extracted 4 times with ether. The organic extracts were washed with water, dried over Na_2SO_4 and evaporated to dryness. The residue was fractionated by flash chromatography over silica gel (ether-benzene 1:1) yielding 40 mg of 11, λ max (MeOH) 326, 234 nm; m/e 332 (M+, $C_{18}H_{16}N_2O_2S_2$: found 332.0641, requires 332.0653); ^{1}H-NMR (acetone-d_6): δ (ppm) 7.65 (2H, bs, OH, OH'), 6.6-6.4 (6H, m, aromatic protons), 5.3 (2H, bs, NH, NH'), 3.82 (1H, ddd, J=9.2, 4.1, 3.1 Hz, H-3'), 3.71 (1H, dd, J=12.4, 5.0 Hz, H-3a), 3.60-3.45 (2H, m, H-2 and H-3b), 3.33 (1H, dd, J= 12.7, 4.1 Hz, H-2'a), 3.12 (1H, dd, J=12.7, 3.1 Hz, H-2'b); ^{13}C-NMR (DMSO-d_6): δ (ppm) 148.88, 148.21 (2xs, C-7, C-7'), 135.40, 133.68 (2xs, C-10, C-10'); 116.45, 116.24 (2xd, C-8, C-8'), 115.59, 114.69 (2xs, C-9, C-9'), 113.18, 112.78, 112.56, 112.49 (4xd, C-5, C-5', C-6, C-6'), 50.67 (d, C-3'), 42.77 (t, C-3), 42.41 (d, C-2), 27.36 (t, C-2').

ACKNOWLEDGEMENTS

This work was supported by a grant from Ministero della Pubblica Istruzione (Rome). One of us (O. C.) wishes to thank Lawrence M. Gelb Research Foundation (Stamford, USA) for a fellowship.

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